History of U.S. Military Contributions to the Study of Diarrheal Diseases

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Diarrhea, a scourge upon humanity since preliterate times, has been the particular nemesis of military forces. The Armed Forces of the United States have been in the forefront in the diagnosis, treatment, and prevention of diarrheal illness. U.S. military scientists and physicians implemented the first mandatory typhoid inoculation program, contributed to advances in water chlorination, and pioneered the use of antibiotics for typhoid fever. U.S. Navy physicians refined the intravenous treatment of cholera, reducing the death rate from 20% to less than 1%. Their studies of electrolyte and fluid balance in cholera, and the subsequent development of oral rehydration therapy for cholera and other diarrheal illness, have saved millions of lives worldwide. U.S. Army researchers refuted the desquamation theory of cholera pathogenesis, isolated the cholera exotoxin, and developed improved cholera vaccines. U.S. Army and Navy researchers pioneered the use of antibiotics for the treatment of typhoid fever, made major contributions to the treatment of dysentery, developed algorithms for the treatment of traveler's diarrhea, and continue active development of traveler's diarrhea and dysentery vaccines. U.S. military diarrheal research has directly contributed to the welfare of hundreds of millions of people.

Introduction

Infectious diarrhea, although neither glamorous nor exotic, has been the constant companion of human society since before recorded history. The Greeks, Romans, and ancient Israelites were all acquainted with the disease and independently made recommendations about the best way to prevent its occurrence and treat its various manifestations. Although diagnosis was straightforward, effective treatment was hampered until comparatively modern times by the lack of a solid understanding of microbiology and pathophysiology. One of the great, if relatively unheralded, successes of modern medicine has been the dramatic reduction in infectious diarrhea-related deaths.

The U.S. military has had a deep interest in the causes, prevention, and treatment of infectious diarrhea since the foundation of the republic. As early as the Revolutionary War, General George Washington, dismayed at the general uncleanliness of his camps, issued several general orders mandating sanitation measures among the troops. In particular, he exhorted the officers and men to refer to the Mosaic code of Deuteronomy, which required latrine placement a certain distance outside the campground. Baron von Steuben, who drilled the Continental Army at Valley Forge, issued specific regulations for latrine placement; he emphasized, as did others, that health and cleanliness were not medical issues but rather matters of military discipline and thus the responsibility of the commanding officer. For example, Dr. James Tilton, a surgeon in the Continental Army, wrote in a treatise on military medicine,

> It may seem strange at first view, that I should call upon commanding officers to take care of the health of the men under their command, or that I should expect they would pay any regard to sickness incident to an army. I hope, however, in the sequel to shew that upon them especially depend the health and comfort of the soldiers, and that the medical staff are only to be regarded as adjutants, in the recovery of the sick.[1]

Unfortunately, lessons learned in the War of Independence were soon forgotten. By the Mexican War of 1848, camp hygiene was universally unsatisfactory; not surprisingly, for every American death caused by injuries there were seven attributable to disease, chiefly dysentery.[2] Matters were not significantly improved in the American Civil War, in which 44,558 Union soldiers died of diarrhea or dysentery (compared with 110,070 combat-related deaths). Recognition of the inadequacy of preventive medicine measures led to the formation of the United States Sanitary Commission, a civilian organization dedicated to improving the health and welfare of Union soldiers. Surgeon General W.A. Hammond founded the Army Medical Museum (forerunner of the Armed Forces Institute of Pathology), to provide U.S. military medicine with a solid foundation of knowledge and experience. Another famous Civil War physician, J.J. Woodward, wrote a comprehensive treatise on camp diseases and was the first microbiologist to use the relatively new technology of photography.[3] Interestingly, he described the ulcerative changes associated with Peyer's patches in the small bowel of typhoid fever patients, although he did not appreciate their significance from a bacteriological standpoint. A Confederate surgeon, Joseph Jones, even observed typhoid bacilli in the mesenteric lymph nodes of typhoid fever patients, anticipating by decades the work of Eberth.[4] However, because the miasmatic theory of disease still dominated most medical thinking, these findings were relatively unappreciated.

George Miller Sternberg, Surgeon General of the U.S. Army, helped usher in the modern era of diarrhea research in the U.S. military. Although principally remembered as the founder of the Army Medical School (forerunner of the Uniformed Services University of the Health Sciences), he also established the Reed-Vaughan-Shakespeare board on typhoid fever during the Spanish-American War. The board's report on the effects of typhoid fever on U.S. military personnel, and the disorganized and ineffective medical care of the time, spurred Sternberg to establish

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tropical disease boards in the new American colonial possessions (Philippine Islands, Cuba, and Puerto Rico), as well as prompting a strong research interest in the U.S. Army in the prevention and treatment of infectious diseases in general. Therefore, by the beginning of the 20th century, U.S. military physicians and researchers were poised to make major contributions in the field of infectious diarrhea.

Cholera

Asiatic cholera once was a phrase that terrorized the entire world. The Gram-negative bacillus *Vibrio cholerae* lives in brackish water and causes epidemics of severe, watery, rapidly fatal diarrhea. Stricken patients produce gallons of rice-water stools, weakening before one's eyes. The volume of diarrhea can reach 800 mL per hour (30 L per day) and can cause prostration and death in hours to days. Death rates among untreated cholera patients exceed 60%; before 1960, 20 to 30% of patients died even with medical treatment. The defeat of cholera as a public health menace is very largely attributable to the efforts of U.S. military physicians and scientists.

U.S. Military Significance

Believed to have originated in the Ganges River Delta, Asiatic cholera was first reported by the British military in 1770. In 1817, the first cholera pandemic spread from India eastward to Japan and westward to the Mediterranean Sea. Successive pandemics struck Europe and the Americas in 1829, 1849, 1852, and 1863, killing hundreds of thousands.[5] O'Shaughnessy (1832) had suggested intravenous therapy, and Latta (1832) had actually tried it with some limited success, but the medical profession rejected it until Rogers revived intravenous therapy in 1921. Using hypertonic saline solution, Rogers was able to reduce mortality rates from more than 60% to less than 30%, although many patients died from pulmonary edema, which was attributed to acidosis. In most parts of the world, no effective treatment was available at all.[6]

U.S. Military Contributions

The Role of Robert A. Phillips

The U.S. Navy's involvement with cholera began in Cairo, Egypt, during the 1947 cholera epidemic. CDR Robert A. Phillips had recently arrived as the first commander of Naval Medical Research Unit 3 (NAMRU-3). Following Pasteur's dictum "chance favors the prepared mind," Phillips, a physician and laboratory researcher, had been prepared for this outbreak by previous studies at the Rockefeller University in New York, where he had devised the copper sulfate method to measure the specific gravity of blood as a correlate for shock severity among trauma victims.[7] Phillips determined that choleraic stool was approximately isotonic with blood except for containing significantly more sodium bicarbonate (HCO_3^- concentration of 40 mEq/L). The excess stool bicarbonate explained the plasma acidosis of cholera patients (pH 7.2 in severe cases). Phillips noted the lack of protein in the stool, which indicated no disruption of the gut mucosa, and concluded that all of the pathological features of the disease could be explained by the diarrhea alone. This conclusion anticipated the discovery of the cholera toxin by a decade.

Phillips then attempted intravenous resuscitation using a slightly hypertonic saline solution (174 mEq/L Na^+ with 3.4 mEq/L K^+). He calculated the base deficit and gave infusions of sodium bicarbonate to normalize blood pH, which reduced the case fatality rate for severely ill cholera patients from 20% to 7.5%, a remarkable achievement that was largely overlooked at the time. In 1958, Phillips, then a Navy captain and commanding officer of the newly reactivated NAMRU-2 in Taipei, offered his assistance to the Thai Ministry of Health after hearing of a cholera outbreak in Bangkok. Phillips, his executive officer LCDR Raymond Watten, and Francis Morgan, in collaboration with Y.N. Songkhla and B. Vanikiati, confirmed the previous Cairo intravenous therapy results.[8] They also invented the Watten cholera cot, an Army cot with a hole under the buttocks and a rubber funnel to allow the copious choleraic stools to drain down for accurate measurement and sanitation. (Phillips dubbed their team the Order of the Perforated Pad.) Phillips and the NAMRU-2 teams traveled to Dacca, Manila, Saigon, Sarawak, Indonesia, and South Korea, teaching the local physicians and refining their technique. By 1966, the case fatality rate for cholera treated with the Navy method of intravenous therapy was 0.6% (reduced to zero with expeditious treatment).

After the 1958 outbreak, Phillips proposed that the United States should field a team of researchers to Bangkok in anticipation of future outbreaks. In 1959, a team of Army investigators traveled to Bangkok to join Phillips' work on cholera, together with investigators from the National Institutes of Health and Jefferson Medical College. MAJ W.R. Beisel and CPT E.J. Gangarosa used the Walter Reed Army Institute of Research (WRAIR) Crosby capsule[9] to biopsy the small intestine of cholera patients and demonstrated normal mucosa.[10] Dr. Robert Gordon (National Institutes of Health) used intravenous radioactive polyvinylpyrrolidone (povidone) to confirm normal gut permeability.[11] These studies proved that cholera was not an invasive disease.

Phillips had long wondered whether oral rehydration therapy for cholera was possible, because most patients would never have access to costly intravenous therapy. In 1961, Phillips and LCDR Craig Wallace showed that patients in Manila were able to orally absorb potassium and bicarbonate but not sodium or chloride. To see whether lower sodium concentrations might permit water absorption, Phillips and Wallace replaced some of the sodium with glucose, to create an isotonic solution with lower electrolyte levels. To their surprise, sodium and water absorption increased in direct proportion to the concentration of glucose; patients briefly ceased having diarrhea, but it resumed as soon as administration of the solution was stopped. In 1962, Phillips and Wallace, together with Dr. Graham Bull of the British Postgraduate Medical School of London, returned to Manila to test their "oral electrolyte cocktail" among 30 patients. Although 25 patients recovered, 5 died of pulmonary edema. The investigators subsequently learned that the cocktail had been accidentally made hypertonic because the glucose had been given in addition to the normal saline instead of replacing it. The glucose had apparently pumped the excess sodium load into the patients and caused fluid overload. These setbacks temporarily halted progress toward oral therapy.

Phillips became director of the Pakistan-SEATO Cholera Research Laboratory in Dacca in 1965. Norbert Hirschorn, a newly

qualified physician doing his military duty, was sent to Dacca by the National Institutes of Health. During the cholera outbreak of 1966, he was concerned that the hospital would run out of intravenous therapy supplies and he asked Phillips about the possibility of oral rehydration. Phillips reportedly closed his office door, charged Hirschorn with secrecy, opened his safe, and gave him the data from the Manila experiments of 1962. Hirschorn concluded that a truly isotonic solution would avoid the hypernatremia seen in 1962; Phillips agreed to a trial but required that Hirschorn give intravenous hydration first and then "sleep with the patients" to avoid any untoward events. In fact, the patients recovered with far less intravenous fluid than usually needed. The investigators went on to show that glucose and galactose each could stimulate sodium and water absorption but fructose and maltose did not, demonstrating the existence of glucose- and galactose-mediated sodium pumps,[12–14] which could be used to counteract the cholera-induced sodium and water losses. Other researchers subsequently developed packets of salts (oral rehydration salts)[15] and distributed them in the community, so that most cholera cases could be treated locally and cheaply. It was then discovered that oral rehydration therapy was beneficial for all types of infectious diarrhea. It is estimated that this advance, using glucose-based oral rehydration therapy to treat diarrheal diseases, saves millions of lives each year. According to the Lancet, "The discovery that sodium transport and glucose transport are coupled in the small intestine, so that glucose accelerates absorption of salt and water, was potentially the most important medical advance of this century."[16]

Isolation and Characterization of Cholera Exotoxin

Richard Finkelstein, a civilian working at WRAIR, isolated the cholera exotoxin, which he called cholerogen, in 1963.[17] The same year, at the Thailand-SEATO Medical Research Laboratory, he and C. Benyajati gave an infusion of choleragen to two healthy human volunteers. A dose four times that producing diarrhea in rabbits produced 18 L of diarrhea. Supposedly, Finklestein dared not put his name on the article for fear of the wrath of the U.S. Army, but Benyajati published the study in 1966.[18] Later in Bangkok, Finkelstein began the work of purifying the cholera toxin, a task he ultimately completed at Southwestern Medical School in Dallas, Texas, with Joseph LoSpalluto. They showed that there were two components (A and B), both susceptible to immune precipitation but only one toxic.[19] The cholera AB toxin is now a standard model for studies of bacterial exotoxins.

Development and Testing of New Cholera Vaccines

An oral cholera vaccine, made of phenol-killed cells, had been in use since the late 1800s, but field trials in the 1960s showed that it had poor efficacy and high reactogenicity and was generally not cost-effective. Since then, the search for a better vaccine has continued. U.S. and Peruvian Army and Navy investigators at the Naval Medical Research Institute Detachment (NAMRID) in Lima, Peru, have studied El Tor cholera (present in Peru since 1991) and cooperated to test several new cholera vaccines. COL Jose Sanchez (U.S. Army) and colleagues at WRAIR tested WC/rBS, a recombinant vaccine of whole *Vibrio* cells plus cholera toxin B subunit, for safety and immunogenicity among U.S. military personnel.[20] At NAMRID-Lima, Sanchez and colleagues showed that, after two doses, efficacy was 86% at 3 months during an epidemic of El Tor cholera with a 2% attack rate.[21] COL David Taylor (U.S. Army) and colleagues, also at NAMRID-Lima, then tested this vaccine in a population of Peruvian children and adults during a 2-year period and found no efficacy against El Tor cholera after two doses but 61% efficacy after three doses.[22] The Department of Defense (DoD) contributed to basic science research into live attenuated cholera vaccines at WRAIR,[23,24] the Armed Forces Research Institute of Medical Sciences in Bangkok,[25] and NAMRU-2,[26] to provide broader strain and biotype immunity.

Summary and Key U.S. Military Contributions

U.S. military investigators clarified the pathophysiology of cholera, refined intravenous therapy to decrease the case fatality rate from 20 to 30% to less than 1%, and developed oral rehydration therapy so that the majority of cholera patients could be successfully treated with oral fluids alone. Oral rehydration salts were shown to be useful for other, more common forms of infectious diarrhea and have become the mainstay of therapy for diarrhea of any etiology. The number of lives saved in the past several decades can be estimated in the millions. Military researchers have been on the front lines of cholera vaccine research; in order of impact, their five most significant contributions to the understanding and management of cholera were the (1) discovery by Phillips, Wallace, and colleagues that isotonic electrolyte solutions containing glucose could be administered orally to rehydrate patients with cholera and other diarrheal diseases (estimated lives saved, hundreds of millions); (2) refinement by Phillips, Watten, and colleagues of intravenous therapy for cholera using isotonic saline solution plus bicarbonate and potassium, reducing the case fatality rate from 20% to less than 1% (estimated lives saved, hundreds of thousands); (3) refutation of the desquamation theory of cholera pathogenesis by Beisel, Gangarosa, and Gordon; (4) isolation of cholera toxin by Finkelstein; and (5) development and testing of improved cholera vaccines by Taylor, Sanchez, and colleagues.

Dysentery

Dysentery is generally defined as an inflammatory enteric disease characterized by fever, abdominal pain, and bloody or purulent diarrhea. The most common causes are *Shigella*, *Salmonella*, and *Campylobacter jejuni*. Although relatively rare in industrialized countries, outbreaks continue every year, generally because of some lapse in sanitation or food preparation. The control of dysentery is one of the major advances of modern medicine.

U.S. Military Significance

Dysentery-type illnesses have had an enormous impact on the course of military operations. From the campaigns of the Persians and the Greeks and the death of Henry V to the debilitating effects on Union soldiers in the Civil War and German troops in North Africa, dysentery has been the nightmare of countless military commanders. Hippocrates seems to have been aware of dysentery and its relationship to unsanitary water; Roman military historians also warned that unsafe water

produced illness among troops. Even today, military forces are at risk whenever basic precautions are not enforced.[27]

Shigella

Shigella species are small Gram-negative rods (easily grown on selective Gram-negative medium) that have traditionally been associated with outbreaks of dysentery, especially during military campaigns. *Shigella* is the prototypical enteroinvasive pathogen; as a general rule, the organism does not penetrate beyond the mucosa and thus only rarely causes bacteremia. Different species predominate in different eras and regions and, although *Shigella dysenteriae* was the original Shiga bacillus, *Shigella sonnei* is currently the most common U.S. isolate.

U.S. Military Contributions

Although Shiga was the first to isolate *S. dysenteriae*, it is not widely known that U.S. Army physicians in the Philippines were among the first to confirm his work, in studies of U.S. troops with bacillary dysentery. Another important advance in *Shigella* research was the demonstration by E.H. LaBrec and Sam Formal, of the WRAIR, that the propensity of *Shigella* for tissue invasion was central to its pathogenicity.[28] In collaboration with various civilian partners, Formal also demonstrated the remarkably low infectious inoculum of *Shigella*,[29] the role of lipopolysaccharide, the molecular basis for pathogenesis, and the existence of Shiga-like toxin in some strains of pathogenic *Escherichia coli*[30]; the latter work helped elucidate the pathophysiology of enterohemorrhagic *E. coli*.[31] U.S. military researchers also demonstrated that loperamide with antibiotics could be used safely in the treatment of *Shigella*-induced dysentery, an important advance in the empiric treatment of diarrhea,[32] and demonstrated the limits of short-duration therapy.[33] WRAIR investigators led early efforts to investigate genetically modified, attenuated, oral vaccines that continue to be investigated as promising vaccines for *Shigella* prevention,[34,35] as well as vectors for other antigens. *Shigella* remains of considerable interest to the U.S. military, as seen in Operation Desert Shield (1990), where it was the second most common bacterial diarrheal pathogen.[36]

Campylobacter jejuni

C. jejuni is the most common bacterial enteric pathogen worldwide. Although in developing countries it is frequently isolated from the stool of healthy persons, in modern societies it is rare except in the setting of acute illness. *C. jejuni* is a microaerophilic Gram-negative rod that is best incubated at 42°C on selective medium (to prevent overgrowth by other, more rapidly dividing organisms). The most common clinical presentation of *C. jejuni* is diffuse enteritis, which may affect the jejunum, ileum, or colon; patients generally present with fever, abdominal pain, and diarrhea. The disease is generally self-limiting, although relapses are occasionally seen. Occasionally *C. jejuni* is associated with Guillain-Barré syndrome, 2 to 3 weeks after the acute infection; approximately 30% of Guillain-Barré syndrome cases are associated with *C. jejuni* infection.[37]

U.S. Military Contributions

Although relatively new, the *Campylobacter* vaccine research program at the Naval Medical Research Center has produced major advances in the understanding of flagellar motility, genetic mechanisms, and animal model development.[38–41] This has led to the first-ever candidate, inactivated, whole-cell and second-generation, recombinant subunit vaccines. In recent years, *C. jejuni* infections have been complicated by the development of widespread quinolone resistance. Some of the earliest reported resistant strains came from Southeast Asia, isolated in the context of joint Thai-United States military exercises. Whereas no *C. jejuni* strains were resistant to ciprofloxacin in 1987 and 1990, 40% were resistant in 1993 and 83% in 1995.[42] Further work from the same group demonstrated the usefulness of azithromycin in the treatment of campylobacteriosis,[43] and azithromycin is now the standard treatment for *Campylobacter*-associated enteritis. The longitudinal nature of the diarrhea studies carried out by Peter Echeverria and colleagues at the Armed Forces Research Institute of Medical Sciences allowed careful characterization of the changing ecology of enteric pathogens in Thailand and demonstrated the usefulness of linking medical research efforts to military exercises and deployments. Similar projects continue, in cooperation with the Indonesian and Egyptian governments, at the NAMRUs in Jakarta and Cairo. Surveillance for antibiotic resistance among enteric pathogens in the Middle East, Southeast Asia, South America, and Oceania continues to provide needed data on changing trends.

Summary of Key U.S. Military Contributions

The key military contributions are (1) extensive and ongoing work on *Shigella* and *Campylobacter* pathogenesis and immunity; (2) continued development of *Shigella* and *Campylobacter* vaccines; (3) treatment algorithms for *Shigella* and *Campylobacter*, including research on the use of loperamide for dysentery and azithromycin for fluoroquinolone-resistant *Campylobacter*; and (4) ongoing surveillance efforts in developing countries to monitor for the emergence of drug-resistant strains of pathogenic bacteria.

Typhoid

Salmonella typhi is an obligate human pathogen. Infection occurs via ingestion of fecally contaminated food or water from an unknowing carrier or an acutely ill patient. Depending on the inoculum, typhoid fever has an incubation period of 7 to 21 days. Bacilli multiply in the small intestine, invade through intestinal lymphatic vessels (causing hyperplasia and necrosis of the lymphoid Peyer's patches), and disseminate systemically. Blood cultures turn positive, with a remittent fever that becomes sustained, lasting 10 days or more, and is associated with malaise, cough, nausea and vomiting, and headache. By the second or third week of illness, abdominal tenderness, distention, and splenomegaly are prominent; during this time, the characteristic "rose spots," an erythematous macular rash typically observed on the trunk, appear for approximately 30% of patients. Diarrhea usually occurs early in the course of the disease and resolves before fever appears. Illness typically lasts 3 to 5 weeks if untreated. Major complications include gastrointestinal hemorrhage, bowel infarction with peritonitis, pericarditis, meningitis, and septic arthritis. Diagnosis is made by isolation of the organism in cultures of blood, urine, stool, or occasionally bone marrow.

U.S. Military Significance

Typhoid has been implicated in the deaths of numerous historical figures, including Alexander the Great and Prince Albert (consort of Queen Victoria). It was a major cause of morbidity during the American Civil War, when it was often confused with malaria (because of its chronicity and nonspecific symptoms). Eberth's discovery of the typhoid bacillus in 1884 paved the way for the scientific study of typhoid fever. The first typhoid vaccines were developed in England and Germany at the end of the 19th century. Public interest in typhoid vaccines was particularly acute in Great Britain, whose troops deployed to the Boer War were falling ill at intolerable rates. Very soon, the U.S. military would face its own typhoid crisis, a hemisphere away.

U.S. Military Contributions

MAJ Walter Reed, a public health physician, was commissioned in the U.S. Army Medical Corps in 1875. After several assignments in the West and Midwest, he was assigned to Baltimore's Fort McHenry in 1890, which afforded him the opportunity to work at Johns Hopkins Hospital with William Welch on the pathology of typhoid fever and the identification of hog cholera bacillus. Reed was promoted to Major in 1893 and became curator of the Army Medical Museum as well as professor of bacteriology and clinical microscopy in the Army Medical School (now the WRAIR).

In 1898, the battleship Maine exploded in Havana Harbor, precipitating the Spanish-American War. Disease was the greatest enemy of the men in the field. Febrile illness seemed to occur frequently in U.S. military camps, although the diagnosis was not clear; typhoid fever, "typhomalarial fever," and typhus were among the diseases considered. Army Surgeon General George Miller Sternberg appointed a board led by Reed to investigate the cause of the disease's prevalence in almost all U.S. Army encampments. The board consisted of Reed, Victor C. Vaughan, an epidemiologist and physiologist from the University of Michigan Medical School, and Edward O. Shakespeare, a bacteriologist and ophthalmologist with experience with cholera outbreaks in Spain. They undertook an investigation of the 15th Minnesota Volunteer Infantry, in which only eight men were healthy, of four officers and 105 enlisted men. Their report, a model of epidemiological investigation, ruled out typhus as a major contributor to illness, demonstrated the difference between typhoid and malaria, and proved that "typhomalaria" did not exist.[44] The Reed-Vaughan-Shakespeare board's maps showed improper siting of latrines and kitchens, and their inspections documented poor camp sanitation. Dr. Reed and the board ordered more thorough disinfections of all men, tents, blankets, clothing, bedding, and equipment of the unit. The initial source of infection was credited to the "notoriously infected water" supply of the city of Minneapolis. An overlooked aspect of Reed's research was his conclusion that asymptomatic carriers could spread typhoid, an insight that would not become widely accepted until the case of Mary Mallon ("Typhoid Mary"), a decade later. Surgeon General Sternberg concluded in 1899 that carriers of typhoid from civilian society to stateside training camps into battlefields contributed greatly to the epidemics in U.S. military camps.

The board's statistics showed that more Spanish-American War soldiers died from training stateside than from fighting overseas. The rate for stateside typhoid admissions per 1,000 soldiers decreased from more than 85 in 1898 to less than 6 in 1900, with the initiation of water sterilization and improved sanitation practices. Unfortunately, history repeated itself with a doubling of the rate in 1901 and persistently high rates in 1902, because of the soldiers' resistance to preventive measures. The board's previous work was often ignored. Shortly before Walter Reed died in 1902, he investigated a typhoid outbreak at Fort H.G. Wright, New York. Reed then reconstructed a familiar history of poor sanitation conditions, human contact, flies, and spread to three other military installations. In the wake of Reed's work at Fort Wright, Surgeon General Robert M. O'Reilly issue of Adjutant General's Circular 62, addressing rules of personal hygiene. An outbreak of fever in the Army camp at Chickamauga, Georgia, in 1902 was investigated by contract surgeon James Carroll and Maj Jefferson R. Kean. The fever was traced to the neighboring village of Rossville, where poor sanitation conditions were noted among civilians.

MAJ Frederick Fuller Russell was sent to Europe to study the new typhoid vaccine of Sir Almroth Wright. Within a remarkably short time, Russell modified the vaccine for subcutaneous use and carried out volunteer studies; this permitted the Army to begin a massive vaccine-production effort and institute mandatory vaccination by 1911 for U.S. Army personnel dispatched to the Mexican border because of the Mexican Revolution. Surgeon General George Torney, O'Reilly's successor, subsequently reported that a vaccinated division of 12,800 men suffered one case of and no deaths from typhoid fever while encamped at San Antonio, Texas. Torney compared these statistics with those compiled by the first typhoid board for a comparable 10,700-man division in 1898, with 2,693 cases and 248 deaths. Torney added that a unit that camped outside Galveston, Texas, in 1911 lacked "sanitary plumbing" but suffered no typhoid casualties, whereas the city's population sustained 192 cases during the same period, despite modern plumbing. After the entry of the United States into World War I, the U.S. Army had by far the lowest typhoid incidence of any major combatant.[45]

The U.S. military's contributions continued through the antibiotic era. A significant milestone was reached in 1948 with the research by Woodward, Smadel, and colleagues (supported by the Armed Forces Epidemiology Board and WRAIR) into the use of chloramphenicol to treat typhoid,[46] the first antibiotic typhoidal cure in history. Hoffman et al.[47] (NAMRU-2) demonstrated the usefulness of dexamethasone in the treatment of typhoidal delirium, a not-uncommon complication of the disease; this has now become the standard of care. Finally, NAMRU-3, in Cairo, Egypt, demonstrated the usefulness of ciprofloxacin, azithromycin, and third-generation cephalosporins for the treatment of typhoid fever among adults and children,[48–51] which was a major advance, given the increasing prevalence of drug-resistant *S. typhi*. Research into modern typhoid vaccines has also continued.[52,53]

Summary of Key U.S. Military Contributions

The key military contributions were (1) the founding of the Reed-Vaughan-Shakespeare board, a model of epidemiological investigation for subsequent outbreaks of typhoid and other diseases; (2) global vaccination of the U.S. military before World War I; (3) Woodward's use of chloramphenicol to treat typhoid; (4) Hoffman and colleagues demonstrating the usefulness of

dexamethasone in the treatment of typhoidal delirium; and (5) NAMRU-2 and NAMRU-3 research into alternative antimicrobial agents for typhoid fever.

Traveler's Diarrhea

Travelers' diarrhea is a term initially coined to describe a clinical syndrome among civilian travelers from industrialized regions visiting less-developed countries hyperendemic for various enteropathogens. Although less morbid than dysentery, cholera, or typhoid, traveler's diarrhea can be particularly disruptive for both casual tourists and seasoned travelers, or deployed soldiers. U.S. military researchers continue aggressive research into the prevention and management of traveler's diarrhea.

U.S. Military Contributions

Although much of the seminal work describing travelers' diarrhea originated with U.S. students visiting Mexico (described in detail by Kean[54] in the 1960s), military engagements predate this experience and continue to give military and civilian health care providers valuable infectious disease threat data.[55–57] U.S. troops were not spared significant rates of diarrheal illness during combat operations on U.S. soil (the Revolutionary War through the Civil War) or during border wars (the Mexican-American War).[58,59] As early as the Spanish-American War, higher diarrhea rates were observed among deployed forces in developing regions, fulfilling the standard definition of traveler's diarrhea. As U.S. military overseas missions became more common in the 20th century, the travelers' diarrhea syndrome increased in operational importance.

Early military health surveillance occurred before the identification of the major etiologies of traveler's diarrhea. Efforts were linked to hygiene and sanitation control methods, with emphasis on appropriate practices. Regional differences (United States vs. developing foreign region) became more dramatic following sanitation improvements in the United States. Military contributions include the focus on surveillance efforts, with emphasis on hygiene and sanitation control methods.

MAJ Carl Darnall was the first to develop a means to use pressurized anhydrous chlorine to reduce contamination in water.[60] Darnall also developed a filter method applicable to field use. MAJ William Lyster further adapted the process of water chlorination to field use by inventing a method to apply sodium hypochlorite in a cloth bag (the "Lyster bag").[61] These early accomplishments in water chlorination greatly enhanced the safety of water supplies and continue to yield public health dividends for military personnel and civilians worldwide. Despite these pivotal advances, high rates of diarrhea continued to occur, most likely related to exposures to local foods and beverages, as well as fly-borne vectors.

The Commission on Enteric Infections of the Armed Forces Epidemiology Board was chartered in 1949.[62] This board provided valuable direction and extramural funding to support investigations into the etiology and pathogenesis of enteric infections affecting U.S. forces for the next 23 years. Collaborative research between DoD scientists and academic researchers supported by the commission led to important discoveries such as initial experimental evidence of enterotoxigenic *E. coli* (ETEC) in volunteer challenge studies.[63] ETEC was shown to be the most common cause of travelers' diarrhea; the U.S. military has had a leading role in documenting regional variations in colonization factors and the impact on military personnel.[64,65] This knowledge translates into targets for relevant vaccine candidates.

Travelers' diarrhea management has been a particular area of focus for DoD investigators because of the need to rapidly return personnel to functional status in both wartime and peacetime foreign deployments. As noted above, the efforts of Phillips, Wallace, Watten, and many others led to successful oral rehydration strategies for both cholera and noncholera watery diarrhea. Evidence of antibiotic efficacy for the treatment and prophylaxis of traveler's diarrhea prompted military physicians to investigate treatment options for deployed personnel; the results of DoD, randomized, controlled trials have resulted in major clinical practice changes. These findings can be broadly divided into the following three areas: antibiotic selection, regimen selection (number of doses, duration of treatment, and utility of adjunctive loperamide therapy),[66] and regional concerns (such as antibiotic resistance trends). Military investigators demonstrated the efficacy of fluoroquinolone chemoprophylaxis, the efficacy of 3-day and single-dose regimens (for fluoroquinolones and macrolides), and the additive benefit of loperamide in ETEC-predominant regions.[67,68]

The DoD remains a world leader in traveler's diarrhea prevention efforts through vaccine development. In addition to the previously mentioned work in *Shigella* and *Campylobacter*, the DoD actively pursues development of ETEC vaccines.[69,70] WRAIR investigators have purified, cloned, and evaluated immune responses for one of the most prevalent ETEC colonization factors, CS6, as well as refining vaccine antigen production and evaluating innovative delivery methods such as microencapsulation and transcutaneous immunization.[71,72] Field studies investigating vaccines to protect against ETEC infection were initiated with the oral, killed, whole-cell cholera vaccine with recombinant cholera toxin B subunit, on the basis of homology with ETEC heat-labile toxin and evidence of cross-protection.[73] These efforts led to extensive field studies, based at NAMRU-3, of a multivalent, killed, whole-cell ETEC vaccine.[74,75] From early attempts at the Army Medical School in oral and parenteral delivery of killed, whole-cell, typhoid vaccine in 1900–1915 to the use of modern molecular biology methods to develop attenuated strains and recombinant subunit vaccines, using needle-free technologies targeting the mucosal immune system, the DoD continues its leadership in the field of diarrheal disease vaccine development with focused research programs collaborating with academic, industrial, and public health institutions.

Summary of Key U.S. Military Contributions

The key military contributions are (1) advances in the provision of safe water through chlorination, leading to global application as well as field utility (Lyster bag); (2) ongoing work in the development of traveler's diarrhea vaccines; (3) evidence of ETEC as the principal pathogen in traveler's diarrhea; and (4) development of empiric treatment guidelines for traveler's diarrhea.

TABLE I

TIMELINE OF SIGNIFICANT EVENTS FOR THE U.S. MILITARY AGAINST DIARRHEAL INFECTIONS

1778	Baron von Steuben issued sanitary regulations at Valley Forge; Benjamin Rush (Physician General of the Hospital in the Middle Department) wrote "Directions for Preserving the Health of Soldiers," an early American textbook of preventive medicine
1863	Surgeon General W. A. Hammond wrote "A Treatise on Hygiene with Special Reference to the Military Service"
1898	Typhoid Board headed by Maj Walter Reed (with Maj Victor C. Vaughan and Maj Edwin O. Shakespeare) established to improve sanitation in military encampments; proof of the existence of a typhoid carrier state established
1909	MAJ Frederick F. Russell of the Army Medical School developed an improved typhoid vaccine; this vaccine became mandatory (by command order) for all U.S. Army and Navy personnel in 1911
1910	MAJ Carl Rogers Darnall developed a method of water purification using anhydrous chlorine
1915	MAJ William Lyster developed a water purification system using calcium hypochlorite in a linen bag (Lyster bag)
1917–19	U.S. military deployed in World War I, with lowest typhoid incidence of any major combatant
1947	Phillips at NAMRU-3 in Cairo, Egypt, experimented with isotonic saline and bicarbonate intravenous solutions to treat cholera patients; reduced death rate to 7.5%
1948	First specific cure of typhoid fever with chloramphenicol was documented
1958	Cholera in Bangkok; Phillips and Watten of NAMRU-2 in Taipei traveled to Bangkok and began studies; isotonic nature of cholera stools and blood confirmed; excess bicarbonate loss confirmed; refinement of isotonic intravenous therapy; Watten cholera cot invented
1959	Army and civilian investigators joined Phillips in Bangkok; Beisel and Gangarosa proved gut mucosa is histologically normal in cholera; Gordon confirmed no loss of protein in cholera; sloughing theory refuted
1962	Seventh cholera pandemic, termed El Tor, hit Philippines; Phillips and Wallace established NAMRU-2 detachment in Manila and reduced fatality rate further; oral electrolyte cocktail helped 25 but 5 died from pulmonary edema (discovered solution too hypertonic)
1966	Cholera case fatality rate was 0.6% using Navy technique; both oral and intravenous solutions were used; Phillips awarded the Lasker Prize for most significant contribution to medical science by a U.S. physician
1982	Formal et al. demonstrated Shiga toxin production in *Escherichia coli*
1984	Hoffman et al demonstrated efficacy of steroids in typhoidal delirium
1993	Demonstration of quinolone-resistant *Campylobacter* in Thailand by U.S. Army-Royal Thai Army collaborative effort
1994–2004	Demonstration of the efficacy of ceftriaxone, azithromycin, and other antibiotics for resistant *Salmonella typhi*

Conclusions

The U.S. military, in its more than 200 years of development, has made numerous significant contributions to the understanding of the causes, treatment, and prevention of infectious diarrhea (Table I). Key contributions of the U.S. military include (1) development of oral rehydration therapy, one of the greatest medical interventions of the 20th century; (2) advances in the provision of safe water through chlorination, now in global application; (3) demonstration of the existence of the typhoid carrier state by the Reed-Vaughan-Shakespeare board, and other models of epidemiological investigation; (4) the first successful treatment of typhoid fever with antibiotics; (5) elimination of typhoid fever as an operationally significant threat to military forces; (6) demonstration that cholera's pathogenicity is toxin-mediated and not inflammatory; (7) elucidation of the pathogenesis of *Shigella*; (8) ongoing research into vaccines to prevent *Shigella*, *Campylobacter*, and traveler's diarrhea; and (9) refinement of the treatment of traveler's diarrhea with empiric antibiotics and loperamide, and research into regional variations in antibiotic susceptibility and appropriate alternative antibiotic regimens.

By reducing cholera's mortality rate from more than 50% to nearly zero, U.S. naval and military clinical researchers have saved millions of lives and given a gift of lasting value to the world. The principles of cholera therapy have proven effective in other diarrheal diseases and save more millions of lives each year. Other insights into *Shigella*, *S. typhi*, *Campylobacter*, and traveler's diarrhea have led to significant advances in the prevention and treatment of these diseases. The U.S. military can be proud that its investment in basic research has been well justified. However, infectious diarrhea continues to be one of the principal disease syndromes affecting deployed troops. Vaccines to prevent infectious diarrhea may someday eliminate many if not all of these diseases; in the meantime, meticulous attention to camp hygiene, particularly with respect to food and water supplies, remains, as in the days of Moses and Alexander, the most effective method of defense.

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